Dr. Albert B. Sabin
The Children’s Hospital Research Foundation
Elland and Bethesda Avenues
Cincinnati 29, Ohio

Dear Dr. Sabin:

For your information, the following procedure was employed by Dr. Roberts to prepare the antigens used in the recent blindfold test. I have been advised that seven Lansing and five Brunhilde antigens were prepared. I have no information to indicate whether each unknown serum was run against each Lansing and Brunhilde antigen.

"The original suspension of Lansing virus possessed LD₅₀ of 10⁻⁴·⁵ to 10⁻⁵. No virus was lost by protamine precipitation of 4₀°C. A 2-cycle ultracentrifugation and consequent 100 fold concentration resulted in virus preparations with LD₅₀ of 10⁻⁶·⁵ to 10⁻⁷ and contained only 0.35% of the original nitrogen, or a loss of 99.65%. The LD₅₀ was 1.2 x 10⁻¹¹ gm. of nitrogen. These preparations were diluted with an equal volume of saline tested by the flocculation method. Strong reactions were observed at dilutions of 1:160 and 1:320 with a final titer of 1:1280 dilution of the Lansing convalescent monkey serum.

"The original Brunhilde virus suspension gave an LD₅₀ of 10⁻⁴, while the final preparation (50 x conc.) was 10⁻⁵ or 1.5 x 10⁻⁸ gm. of nitrogen. A loss of 99.4% of the original nitrogen was accomplished. Flocculation was observed at 1:320 dilution of the Brunhilde convalescent monkey serum. Cross reactions were observed between the Lansing antigen and the Brunhilde serum at lower dilutions.

"The procedure for preparing the partially purified virus suspension is as follows:

1. Prepare an aqueous protamine solution containing 10 mg. per ml. (room temperature).

2. Prepare the virus suspension by grinding the infected tissue (fresh or frozen) in a mortar and suspend in 0.02 M K₂HPO₄ saline (10% suspension of cotton rat tissue. 20% suspension of monkey tissue).

3. Centrifuge in the large IEC angle head at 4,000 rpm (3,000 x G) for 30 minutes.

4. Collect the supernatant and adjust to pH 7·3. This suspension may be frozen and stored at -20°C or processed immediately.
5. Measure the volume, chill the virus suspension to \(4^\circ\) C. and add the protamine solution (100 mg./ml.) slowly with stirring until 0.5 mg. per ml. of virus suspension has been added.

6. Hold at \(4^\circ\) C. for 30 minutes with occasional stirring. Centrifuge at 4,000 rpm (3,000 x G) at \(4^\circ\) C. for 30 minutes. Decant and collect the supernatant and add protamine solution until an additional 1.5 mg. per ml. of virus suspension has been added (this permits removal of normal components and avoids an excess).

7. Hold overnight at \(4^\circ\) C. and centrifuge at 4,000 rpm at \(4^\circ\) C. for 30 minutes. Collect the supernatant and centrifuge at 40,000 rpm (144,000 x G) in a no. 40 rotor of the Model L Spinco for 90 minutes.

8. Discard the supernatant and dissolve pellets from one head (12 tubes) in 10 ml. of saline. Centrifuge at 15,000 rpm for 15 minutes in the no. 40 rotor.

9. Save the supernatant and centrifuge at 40,000 rpm for 90 minutes. Discard the supernatant and dissolve the pellet in 1/50 of original volume of 0.01 M PO\(_4\)-saline buffered at pH 6.9.

10. Multispeed at 16,000 rpm for 20 minutes before testing.

"With these more purified suspensions, the flocculation test should not be incubated at \(43^\circ\) C. for more than 1\(\frac{1}{2}\) hours."

I thought you might find this information useful.

Cordially yours,

H. M. Weaver
Director of Research